added to the culture.[5] These results suggest that the antibiotic modification is not necessarily a primary self-defence mechanism for all the t-type trichothecene producers, as we have previously discussed.[1]The type A trichothecene producer would have other defence options dispensable with 3-*O*-acetylation. These possible options might include modification or replacement of the drug target ribosome,[6] efficient efflux of the antibiotic by the membrane transporter,[7] and restriction of the membrane permeability against substances in the medium,[8] which might be substantially defective in the type B producer.

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Parallel synthesis and automated data analysis: A versatile decision tool

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Abstract: The problems associated with the use of combinatorial chemistry in lead generation and optimization are discussed. A post-synthesis data evaluation process is described which can cope with large data sets from parallel synthesis effects. It relies on analysis of purity as well as on identification, and can efficiently limit compounds screened to manageable numbers.

Keywords: combinatorial chemistry; parallel synthesis; decision algorithm

Combinatorial chemistry recently emerged as a powerful tool for lead generation and lead optimization. Its growing importance is demonstrated by the observable fast implementation of combinatorial methodologies into all major pharmaceutical and agrochemical companies[1] and is backed by the desire for faster drug discovery cycles. Over the years a variety of combinatorial and automated synthesis techniques have been described.[2,3] Each of these has its own strengths and weaknesses with respect to the support of the various parts of the drug discovery process. For example split-and-mix methods[4,5] allow access to very large numbers of compounds for high-throughput screening, but the necessity for deconvolution and the risk of dealing with false positives are serious drawbacks. The latter is also true for the use of encoded libraries where the structure elucidation process of actives is significantly enhanced by an efficient read-out of the synthesis information encoded by some sort of tag.[6–8] Finally, parallel methods offer the possibility for fast single-compound synthesis,[9] but the process is labour-intensive and generally only a limited number of compounds can be reasonably handled at a time. Considerable advantages of parallel synthesis approaches are that no encoding other than keeping a spatial address in an array format is necessary and that compounds can be synthesized in any amount and by any chemistry either in solution or on a solid support.

Over recent years, improvements in laboratory robotics and data management tools have contributed significantly to the rapid growth of parallel synthesis methods. Some drawbacks in this approach still exist because it is inherent in the standard conditions usually applied that side-product formation or incomplete reaction occurs. However, screening impure products in a lead-optimization phase is undesired. Two solutions to avoid this problem exist: extensive reaction-optimization programs or application of rapid analysis and purification tecniques. Currently the latter strategy is of high interest[10] and in this paper our efforts concerning this topic are reported.

We favour an approach that deals with sample follow-up which is designed to yield pure compounds of known, as well as unknown structure. The thesis is that any novel chemical entity constitutes an element of molecular diversity and should be tested in a high-throughput random screen. To achieve this goal we rely on a two-step process which starts with an analytical LC/MS run of an aliquot of each sample followed by automatic data interpretation and sample categorization. Preparative purification is

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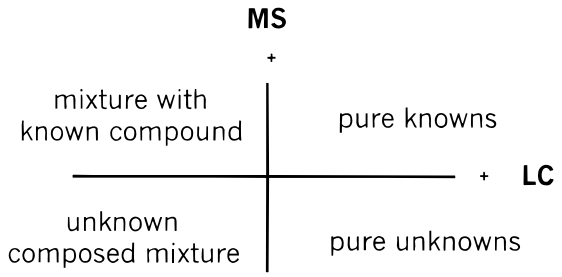

Figure 1. The four categories from LC/MS runs.

ultimately done for samples not fulfilling previously set purification criteria. Central in this process is a decision algorithm which is based on the analysis of the orthogonal information content (purity and identity) obtainable from LC-MS runs. Figure 1 shows that four categories are possible by combining these information axes in a logical way. Each sample is hence assignable to one of the quadrants.

An evaporative light scattering detector (ELSD) is used for purity assessment because, for a given molecular mass range, the detector response is more linear with concentration than UV absorbance.[11] Peaks can hence be sorted in a semi-quantitative way according to weight, and the number of peaks for collection can be efficiently reduced by applying a threshold value: eg only those peaks are collected for which the estimated yield exceeds the required amounts for biological screening (weight-based collection).

The decision algorithm we use is an in-house development programmed in Visual Basic whose input requirements consist of a sample ID, expected

Table 1. Results file of a subset of a library of 192 isoxazoles

Decision Criteria:
Purity: 80.0% as the sum of 1 Peak(s)
Maximum number of fractions: 3
Minimum relative peak area: 5.0%
Minimum absolute peak area: 0 counts

MS Peak Search Criteria:
Mass range from 200 to 900 Da
Min. area 1000 Min. BPI 5.0%
Scan (80:1500) ES +

Sample ID	*Formula*	*M(calc)*	*Mass 1*	*Mass 2*	*Mass 3*	*Result*	*Sum ELSD*
MD-10020	$C_{20}H_{21}N_3O_4$	367.2				Pure known	92.6
MD-10044	$C_{26}H_{25}N_3O_3$	427.2	278.0	428.1		Mixture with known compound	100.0
MD-10056	$C_{23}H_{25}N_3O_5$	423.2				Pure known	96.9
MD-10068	$C_{25}H_{23}N_3O_3$	413.2	278.0	296.0		Unknown composed mixture	100.0
MD-10080	$C_{25}H_{21}Cl_2N_3O_3$	481.1				Pure unknown with mass = 278.0	80.1
MD-10009	$C_9H_{17}N_3O_4S$	263.1	264.0	274.0		Mixture with known compound	100.0
MD-10021	$C_9H_{17}N_3O_5S$	279.1				Pure known	92.7
MD-10033	$C_{13}H_{25}N_3O_4S$	319.2	320.0	330.1		Mixture with known compound	100.0
MD-10045	$C_{15}H_{21}N_3O_4S$	339.1				Pure known	89.5
MD-10057	$C_{12}H_{21}N_3O_6S$	335.1	322.0	336.0	332.0	Mixture with known compound	93.5
MD-10069	$C_{14}H_{19}N_3O_4S$	325.1				Pure unknown with mass = 206.9	100.0

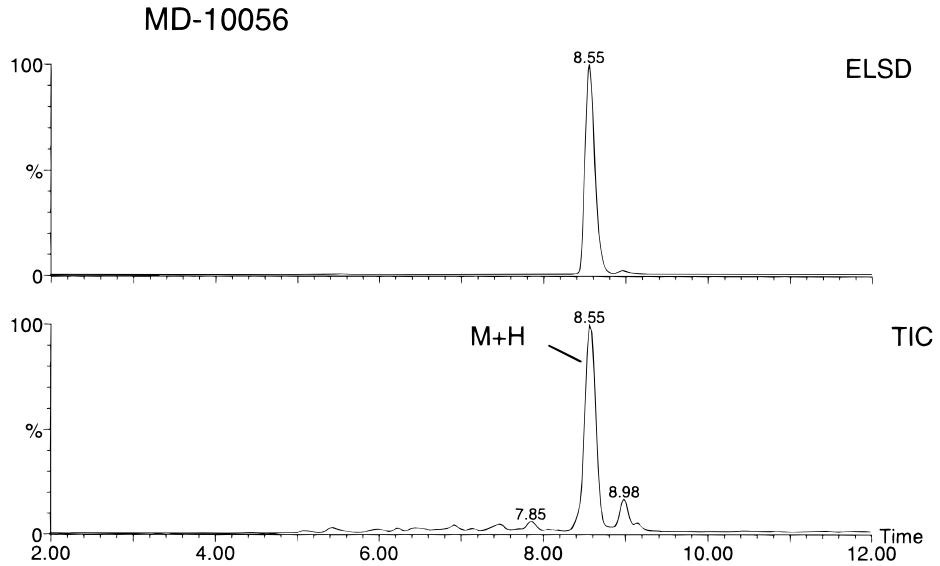

Figure 2. ELSD original and total ion control of compound MD 10056 from Table 1.

molecular mass information and a combined peak table (base peak masses assigned to ELSD peaks). The decision process is based on the analysis of purity (% ELSD area), assignable base peak mass, the number of peaks with the same base peaks (to account for isomers) and minimum relative and minimum absolute peak area of the ELSD signal. An additional criterion which can be applied is the number of fractions one wants to collect for the preparative run.

To demonstrate the decision power of the algorithm a copy of a result file of a representative subset of a library of 192 isoxazoles prepared by solid-phase synthesis[12] and analyzed by analytical LC/MS ELSD is shown (Table 1). It consists of several columns which read out general result information, mass information for triggering preparative fraction collection (mixtures only) and as a quality measure the sum of the ELSD area for the number of peaks used for fraction collection. Analysis of this result file in a spreadsheet program can now efficiently guide the decision process for sample follow-up. For comparison purposes the ELSD signal and the total ion current (TIC) of typical examples of the 'pure known' (Fig 2) and the 'mixture with known compound' (Fig 3) category are shown.

In summary, a post-synthesis data evaluation process has been established which allows us to cope intelligently with the large data sets obtained by analysis of libraries from parallel synthesis efforts. It relies on the analysis of purity as well as on identification criteria and efficiently limits the compounds submitted to screening to manageable and traceable numbers. Contrary to purification efforts, which only focus on the isolation of expected compounds,[13] we think that novel compounds should not be thrown away simply because they are of unknown structure but be used as a source of serendipity in the lead-finding process.

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Commercial development and introduction of DiTera™, a new nematicide

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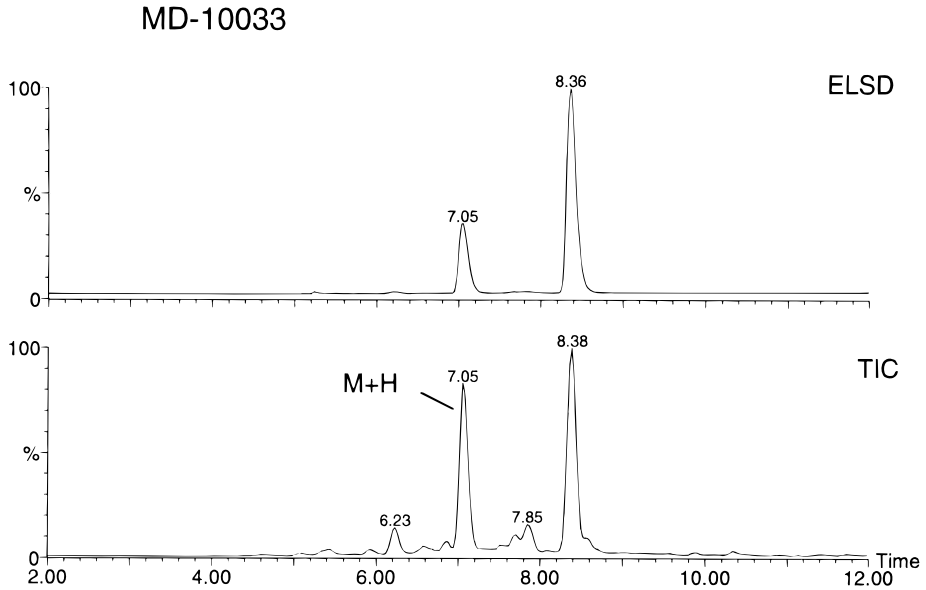

Figure 3. ELSD and total ion control of a mixture with known compound from Table 1.